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Early Ethanol and Water Intake: Choice Mechanism and Total Fluid Regulation Operate in Parallel in Male Alcohol Preferring (P) and both Wistar and Sprague Dawley Rats

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Abstract

The goal of this study was to clarify similar and distinctly different parameters of fluid intake during early phases of ethanol and water choice drinking in alcohol preferring P-rat vs. non-selected Wistar and Sprague Dawley (SD) rats. Precision information on the drinking amounts and timing is needed to analyze micro-behavioral components of the acquisition of ethanol intake and to enable a search for its causal activity patterns within individual CNS circuits. The experiment followed the standard ethanol-drinking test used in P-rat selective breeding, with access to water, then 10% ethanol (10E) as sole fluids, and next to ethanol / water choice. The novelty of the present approach was to eliminate confounding prandial elevations of fluid intake, by time-separating daily food from fluid access. P-rat higher initial intakes of water and 10E as sole fluids suggest adaptations to ethanol-induced dehydration in P vs. Wistar and SD rats. P-rat starting and overall ethanol intake during the choice period were the highest. The absolute extent of ethanol intake elevation during choice period was greatest in Wistar and their final intake levels approached those of P-rat, contrary to the hypothesis that selection would produce the strongest elevation of ethanol intake. The total daily fluid during ethanol / water choice period was strikingly similar between P, Wistar and SD rats. This supports the hypothesis for a universal system that gauges the overall intake volume by titrating and integrating ethanol and water drinking fluctuations, and indicates a stable daily level of total fluid as a main regulated parameter of fluid intake across the three lines in choice conditions. The present findings indicate that a stable daily level of total fluid comprises an independent physiological limit for daily ethanol intake. Ethanol drinking, in turn, stays under the ceiling of this limit, driven by a parallel mechanism of ethanol / water choice.

1. Introduction

In a quest for animal models to study the neurobiological basis of alcoholism, at least seven high alcohol consuming lines of rats were selectively bred over the years [1]. The Indiana

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University alcohol preferring (P) line, derived from the non-selected (“common-stock”) Wistar line, has received the most attention [1,2] and meets all criteria [3-5] proposed for an animal model of alcoholism. The P rat choice drinking of alcohol (10% v/v ethanol solution in water, 10E) vs. free water (see section 2.3) develops over at least three weeks to reach selection criteria of 5 g/kg/day intake and 2:1 preference [6,7]. The existing studies utilize traditional parameters of daily intake and preference to measure choice consumption of ethanol. However, the nature of drinking entangles ethanol consumption with the inevitable intake of water as part of the same solution. Both free water and ethanol solutions together may be subject to regulatory influences governing total daily intake of fluid. Moreover, both are influenced strongly by concurrent feeding. There is a need for a strategy to deal with the confounds of feeding imposed modulation and the entangled nature of water and ethanol drinking.

Based on previous reports, certain questions concerning initial differences that may exist in P versus non-selected rats may be evident even prior to the establishment of choice drinking of ethanol: (a) - Are there differences in water intake between ethanol-naïve P and non-selected rats? (b) - Are there differences seen in initial drinking of an ethanol solution when given as the sole fluid? (c) - Are there differences in daily levels of ethanol, water and total fluid early in choice drinking? (d) - How early, and in what precise mode do the differences between P and non-selected rats appear and evolve? (e) - Do these represent evidence for differential self-exposure to the pharmacological effects of ethanol? (f) - Finally, are there clear behavioral similarities, which would indicate integrating regulatory mechanisms that override line differences, during on-going drinking, which may comprise another level of regulation for ethanol intake?

Consumption of water is known to be subject to a variety of influences [11]. Since ethanol is diluted in water, ethanol intake may result either directly through independent choice or in a secondary fashion through other reasons that directly affect water ingestion. For instance, taste, access schedule, and environmental variables can affect drinking. Food access is particularly important, since 70% to 85% of all fluid consumption naturally occurs in close time association with feeding, i.e., within minutes of food intake [12-14] – prandial drinking. Parameters of feeding and associated drinking vary between rats, and carry individual and line related differences [15-17]. Thus, prandial confounds prevent generation of unbiased, fluid-specific drinking sequences. Prandial drinking has been routinely precluded in numerous studies where animals had access only to fluid, including ethanol, or only to food, in experimental sessions lasting up to several hours. Our early tests indicated that the elimination of prandial confounds was necessary over multiple successive days, to make meaningful comparison of fluid intake parameters between lines. To achieve this, we devised a novel strategy of isolating the food from fluid access within each 24-hour cycle. With this approach, fluids are provided in long sessions overlapping the dark phase of a normal lighting cycle. Free food access in the light phase, separated from drinking sessions by 30-min intervals, allows normal weight gain. This design prevented food deprivation that is known to affect drug and ethanol intake, when body weight is deliberately driven down from the free feeding level before an experiment [20,21]. The arrangement provides an optimal compromise to preclude prandial confounds and allow focused analysis of ethanol vs. water consumption between rat lines in choice conditions.

Our long-range goal is to clarify the CNS circuits and signals that mediate drinking of ethanol and ways of modulating such signals to control its intake. Accordingly, an additional motivation for this work was to gain knowledge on ethanol intake with a very high level of temporal resolution to relate, at a later stage, on-going drinking behavior to causal spatial-temporal patterns of single-neuron activity within specific circuits of the mesolimbic system. An extended effort to develop new instrumentation and evolve the protocol has been

presented in preliminary reports [8-10]. Drinking was studied in precise detail using new lick-sensing spouts and software, in lab cages optimized for concurrent recording of neural activity in free-behaving rats.

The aim of the present study was to characterize early drinking of ethanol, water and total fluid by P rat, with Wistar and Sprague Dawley (SD) non-selected albino rats serving as comparison lines. Wistar is the parental line of the P rat whereas SD is a non-related line, yet both have shown previously similar levels of ethanol intake under certain conditions [18,19]. Our protocol followed the general timeline of a drinking test used for P-rat selective breeding. Initially water, and then 10E, was available as the sole fluid. Last, 10E and water were available concurrently. The instrumentation yielded an integrated body of information on daily intake as well as temporal parameters of drinking ethanol and water. The analysis of drinking patterns and parameters of ethanol and water bouts in P, Wistar and SD rats are presented in our concurrent report. Here we present data on daily intake in the three lines of rats across the phases of the experiment. A preliminary hypothesis was that selective breeding results in consistently higher ethanol intake even during early access to ethanol, while combined levels of ethanol solution and water intake in choice conditions may be subject to a universal systemic regulation of total fluid intake.

2. Material and methods

2.1 Animals

Adult male P rats (59th-60th generation, n=10, Indiana U., IN) weighed 200 g, and Wistar (n=8) and Sprague Dawley (SD, n=14) rats (both from Harlan, IN) weighed 325-350 g upon arrival. P rats were double-housed until growing to 325–350 g, and from this weight all animals were single-housed, in filter-ventilated cages (Thoren Caging System, Inc., Hazleton, PA) at $22 \pm 1.5^{\circ}$ C and $42 \pm 8\%$ humidity under normal lighting cycle (“OFF”=18:00, “ON”=06:00) with food and water ad lib. Body weights were measured once a week until the start of experiment. All animals entered the study upon reaching the weight of 400 g. Experimental procedures were approved by the Institutional ACUC and are in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

2.2 Apparatus

Plexiglas cages measured $40 \times 35 \times 35$ cm (L $\times$ W $\times$ H) and were located in separate light-insulated, sound-attenuated and individually ventilated cubicles. Each cage contained a house light and a fan on top and two spouts, located symmetrically on the sidewalls, 6 cm from the back corners, and 4 cm above the floor. Spouts, custom made in the laboratory, required insertion of the tongue down into a spout well to obtain fluid. Lick movements were monitored by an infrared beam-photosensor setup. Fluids were delivered into the spouts at a rate of 90.25 μ l/sec with model R-E syringe pumps (Razel Scientific Instruments, St. Albans, VT). The data collection and instrumentation control software (Neuroplex, Inc., Winston-Salem, NC) provided monitoring, execution and recording of each lick and fluid delivery at one millisecond resolution.

2.3 Experimental design

The experiment consisted of three test phases (periods). (1) - Water phase: access to 100% tap water alone, as the sole fluid, with no additives (free water, water), for 5 days. (2) - Ethanol phase: access to 10% v/v solution of ethanol in tap water (10E) alone, as the sole fluid, for 5 days. (3) - Choice period, also called ‘ethanol / water choice’: access to concurrent 10E and free water for 12 days. In sole fluid conditions, on day 1 fluid was accessible at both sides, on day 2 at the left side only, on day 3 at the right side only, and so on. In choice conditions, on day 1 water was accessible at the left side and 10E at the right,

on day 2 access sides were reversed and fluid presentation continued with daily side alternation. Phases of experiment followed each other with no interruptions, each animal passed through all 22 sessions.

2.4 Procedures

2.4.1 Adaptation—Before the experiment, animals went into experimental cages for eight-hour exposure on each of three successive days. In these pre-runs, animals stayed in experimental cages from 08:30 to 16:30, with isolating cubicles open, lights in the experimental room “ON” and ad-lib food placed on the cage floor but no fluid access and no data collection. Between and after the pre-runs animals stayed in their home-cages.

2.4.2 Immediate preparation—On the day before the start of experiment, at 17:00 animals were water deprived a single time for 24 hours. On the 1st day at 08:30, animals went into experimental cages with food ad-lib and no fluid. At 16:30, food was removed; at 17:00, the experiment began.

2.4.3 Fluid access—Daily 15-hr access to fluid lasted from 17:00 to 08:00, under normal lighting cycle (“OFF”=18:00, “ON”=06:00). 10E was prepared in tap water from 95% v/v ethanol (Sigma-Aldrich, St. Louis, MO), 24 hours in advance. Spouts were loaded with 0.1 cc unit volumes of fluids at the start of access. Fluids were freely available from spouts; licks were monitored constantly. Every time when licks into a spout reached 32 (the number of licks to consume 0.1 cc in the utilized setup), the lick counter was reset and the delivery started of the next 0.1 cc unit volume into the respective spout. Licks lasted typically 175 – 180 msec in all groups, occurring at an average frequency of ~5.6 Hz, and it would take roughly 5.7 sec to consume a 0.1 cc of fluid. Rate of evaporation from spouts in the utilized setup was evaluated. Evaporation was compensated by deliveries of 10 µl every 600 sec of no licking, which kept a volume in the spout close to what was left by a rat after the previous completed lick. Hence, every consumed 0.1 cc was accounted for, and fluid was always in the spout. Precise timing of licks and coordinated fluid deliveries allowed smooth generation of the spontaneous drinking behavior by an animal.

2.4.4 Feeding—From 08:00 to 17:00, animals remained in experimental cages with no fluid access and no data collection. Animals were weighed on Mon-Wed-Fri between 08:00–08:30 when cages were serviced. Food was available for 8 hours on the cage floor, the amount of 42-52 g/kg/day given at 08:30 and leftovers - at least 0.5 g and usually no more than 3 g - removed at 16:30. Separation of food from fluid access with two 30-min intervals removed prandial confounds and rendered pure drinking behavior for an evaluation. Feeding conditions appeared adequate based on the reported body weight gain of up to 32 g from the starting level of 400-424 g, over 3-4 weeks under 24-hr concurrent access to 10E, water and food [14-16]. The regimen in our study kept body weight close to the level at the start of experiment, preventing loss and supporting a gain of up to ~10% by the completion of the experiment. Over the experiment, the average body weight was 428 g.

2.5 Data preparation and statistical analysis

Every day a dedicated data file was collected from each animal, with total counts of, and one-msec-precise timestamps for each, of the licks and 0.1 cc fluid drops. For this report, the drop counts were converted into daily intake levels. Analyses of drinking patterns are presented in the concurrent publication. Body weights were interpolated for the days between direct measurements. The calculated parameters were daily intake levels of free water, 10E, pure ethanol, total water and total fluid. Total fluid is a sum of selected free water and of 10E; total water is a sum of selected free water and of water part of 10E. Thus for the water phase, parameters of total fluid, total water and free water are the same, as in

Fig. 1: 1-ABC. Ethanol preference is a percentage of total fluid accounted for by 10E. When the difference in a parameter value between Wistar and SD rats was quantitatively minor and statistically insignificant, the groups were averaged into a single non-selected group (W +SD) to enhance comparison with the P-rats. The intake parameters were analyzed by t-Test or individual one-way ANOVA, applied to a pair of values as necessary. To compensate for influence of side bias, alternation of an access side and day-to-day variation of intake level in choice conditions, intake data were averaged over two-day intervals – days 1+2, 3+4, etc., yielding a series of six values for each group over the 12 days of the choice period. The results are expressed as mean values $\pm$ SEM with significance set at $p < 0.05$.

3. Results

3.1 Total fluid intake

Total fluid consists of free water in water phase, of 10E in ethanol phase, and of the sum of selected free water and 10E in choice period. Total fluid level of P rat during water phase (Fig. 1: 1-ABC) was greatest, as compared to the Wistar [$F(1,88) = 4.00$, $p < 0.05$], SD [$F(1,118) = 9.63$, $p < 0.01$] and W+SD groups [$F(1,157) = 9.90$, $p < 0.01$]: 65 ± 4 ml/kg vs. respectively 54 ± 3 , 53 ± 2 and 54 ± 2 ml/kg. During ethanol phase, total fluid decreased from the water phase in P [$F(1,98) = 4.18$, $p < 0.05$] and non-significantly in other rats - Fig. 1: 2-A vs. 1-ABC. Between groups, total fluid of P exceeded that of SD [$F(1,117) = 6.11$, $p < 0.02$] and of W+SD rats [$F(1,157) = 4.29$, $p < 0.05$]. During choice period, total fluid increased from the ethanol phase in P [$F(1,108) = 42.92$, $p < 0.0001$], Wistar [$F(1,86) = 7.38$, $p < 0.01$], and SD rats [$F(1,151) = 38.16$, $p < 0.0001$] – Fig. 1: 3-A vs. 2-A. Total fluid of choice period exceeded levels of water phase in Wistar [$F(1,86) = 4.68$, $p < 0.05$] and SD rats [$F(1,152) = 21.6$, $p < 0.0001$] – Fig. 1: 3-A vs. 1-ABC. Total fluid levels during choice period were similar for P, Wistar and SD rats, and for all three were equal to the P rat level of the water phase.

3.2 Total water intake

During the ethanol phase, total water (the water part of 10E) decreased from the water phase in P [$F(1,98) = 11.69$, $p < 0.001$], Wistar [$df(39)$, $t = 2.38$, $p < 0.05$], SD [$F(1,136) = 16.66$, $p < 0.0001$], and W+SD rats [$F(1,216) = 14.77$, $p < 0.001$] – Fig. 1: 2-B vs. 1-ABC. The values became respectively 51 ± 1 , 47 ± 3 , 46 ± 1 and 47 ± 1 ml/kg. The water part of 10E in Wistar and SD rats was below their intake levels of water phase, in agreement with findings of reduced body hydration in non-selected rats during forced ethanol drinking [25,26]. Between groups, the water part of 10E in P rat exceeded that in SD [$F(1,117) = 6.11$, $p < 0.02$] and W+SD rats [$F(1,157) = 4.29$, $p < 0.05$] – Fig. 1: 2-B. At the same time, the water part of 10E during ethanol phase in P corresponded to the water intake by ethanol-naïve non-selected rats, which was considered normal hydration status. Indeed, P rat water level during ethanol phase was close to levels found in water phase for Wistar [$F(1,88) = 1.15$, $p = 0.28$] and SD rats [$F(1,118) = 1.11$, $p = 0.29$] – Fig. 1: 2-B vs. 1-ABC.

Total water during the choice period (a sum of selected free water and water part of selected 10E) exceeded levels of ethanol phase in P [$F(1,108) = 61.88$, $p < 0.0001$], Wistar [$F(1,86) = 10.54$, $p < 0.01$] and SD rats [$F(1,151) = 69.07$, $p < 0.0001$] – Fig. 1: 3-B vs. 2-B. The important finding is that P consume more water than Wistar and SD rats both initially, in the water phase, when ethanol-naïve and total water equals total fluid, and under access to 10E as the sole fluid - Fig. 1: 1-ABC and 2B. Total water during choice period for Wistar and SD became close to the P rat level of water phase – Figs. 1: 3-B vs. 1-ABC. Choice conditions reduced differences in water intake between P, Wistar and SD rats.

3.3 Ethanol intake

During ethanol phase, ethanol intake in P rat was 4.5 ± 0.1 g/kg, as compared to 4.1 ± 0.1 g/kg in SD [F(1,117) = 6.11, $p < 0.02$], 4.1 ± 0.1 g/kg in averaged W+SD [F(1,157) = 4.29, $p < 0.05$], and 4.1 ± 0.2 g/kg in Wistar rats - Fig. 1: 2-D. During choice period ethanol intake decreased from the ethanol phase in P to 3.6 ± 0.1 g/kg [F(1,108) = 21.82, $p < 0.00001$], in Wistar to 3.1 ± 0.2 g/kg [F(1,86) = 9.91, $p < 0.01$] and in SD rats to 2.2 ± 0.1 g/kg [F(1,151) = 116.08, $p < 0.00001$] - Fig. 1: 3-D vs. 2-D. Choice ethanol intake in P exceeded that in Wistar [df(106), $t = 1.96$, $p < 0.05$] and SD rats [F(1,142) = 49.14, $p < 0.00001$], and in Wistar intake was greater than in SD rats [F(1,130) = 11.62, $p < 0.001$] - Fig.1: 3-D. The important finding was that P immediately demonstrate elevated ethanol intake relative to Wistar and SD rats, during both the ethanol phase and the choice period.

3.4 Gradual changes of intake in sole fluid conditions

During the water phase, intake of each group showed a slight tendency to decrease over days - Fig. 2. In P and Wistar rats, daily alternation of local peaks and troughs of intake started with the day 1 peak. In the SD rat, intake decreased from day 1 to 4, then increased to day 5. P rat water intake exceeded Wistar and SD levels for four days, and on day 5, water intake was 70 ± 6 , 61 ± 9 and 54 ± 4 ml/kg in P, Wistar and SD rats, respectively. During the ethanol phase, in P and Wistar the trends were for increased intake, but in SD rats for decreased intake - Fig. 3. In P and SD rats, daily alternation of intake peaks and troughs started with the peak on day 1. In Wistar rats, intake increased from day 1 to 3, then decreased to day 5. P rat exceeded level of ethanol intake in other groups for four days, and on day 5, intake was 4.8 ± 0.2 , 4.1 ± 0.5 and 4.0 ± 0.2 g/kg respectively in P, Wistar and SD rats.

3.5 Changes of free water and ethanol intake in choice conditions

3.5.1 Free water—Intake of free water during choice period decreased vs. the level of water phase in P [F(1,108) = 113.71, $p < 0.0001$], Wistar [F(1,86) = 53.56, $p < 0.0001$] and SD rats [F(1,152) = 90.22, $p < 0.0001$] - Fig. 1: 3-C vs. 1-ABC. Intake of free water during the choice period also was below the level of 10E-bound water during the ethanol phase in P [F(1,108) = 190.54, $p < 0.0001$], Wistar [F(1,86) = 38.32, $p < 0.0001$] and SD rats [F(1,151) = 51.51, $p < 0.0001$] - Fig. 1: 3-C vs. 2-B. At the transition to choice period, intake of free water was below the end level of the water phase in P [F(1,18) = 40.98, $p < 0.00001$], Wistar [F(1,14) = 4.91, $p < 0.05$] and SD rats [F(1,26) = 8.76, $p < 0.01$] - Fig. 4: days 1-2 vs. Fig. 2: day 5. From the start to the end of choice period, intake of free water decreased further in P [df(9), $t = 2.43$, $p < 0.05$], Wistar [df(7), $t = 2.52$, $p < 0.05$] and SD rats [F(1,26) = 5.10, $p < 0.05$] - Fig. 4: days 11-12 vs. 1-2.

3.5.2 Ethanol—At the transition to choice period, ethanol intake was below the end level of the ethanol phase in P [F(1,18) = 10.37, $p < 0.01$], Wistar [F(1,14) = 6.76, $p < 0.05$] and SD rats [F(1,26) = 24.35, $p < 0.0001$] - Fig. 5: days 1-2 vs. Fig. 3: day 5. P rat ethanol intake at the start of choice period (Fig. 5: days 1-2) was immediately greater than both in Wistar [df(16), $t = 2.01$, $p < 0.05$ one-tail] and SD rats [F(1,22) = 8.57, $p < 0.01$]: 3.5 ± 0.4 versus 2.3 ± 0.6 and 2.1 ± 0.3 ml/kg respectively. A gradual elevation during choice period of P rat ethanol intake was illustrated by averaging data over the 4-day epochs: 1-4, 5-8 and 9-12, the respectively increasing values were 3.4 ± 0.2 , 3.6 ± 0.3 , and 3.8 ± 0.2 g/kg [df(19), $t = 2.41$, $p < 0.05$ vs. the days 1-4]. By the end of choice period, ethanol intake exceeded starting levels in Wistar [df(7), $t = 3.71$, $p < 0.01$] and SD rats [df(13), $t = 2.23$, $p < 0.05$] - Fig. 5: days 11-12 vs. 1-2.

3.6 Total water and total fluid intake in choice conditions

Total water and total fluid during choice period (Figs. 6, 7) across groups showed only minimal change, with a marginal drop in P-rat [df(9), $t = 2.25$, $p < 0.05$ one-tail for both parameters]. The levels were similar at the start, nearly identical by the end, and during choice period, total fluid intake by all groups remained very close to the P rat level during the water phase (65 ± 4 ml/kg).

3.7 Development of ethanol preference

P rat exhibited a greater initial ethanol preference vs. Wistar [df(16), $t = 2.03$, $p < 0.05$ one-tail] and SD rats [$F(1,22) = 11.92$, $p < 0.01$] - Fig. 8: days 1-2. Ethanol preference increased during choice period in P [df(9), $t = 2.10$, $p < 0.05$ one-tail], Wistar [$F(1,14) = 7.51$, $p < 0.02$] and SD rats [df(13), $t = 2.83$, $p < 0.02$] - Fig. 8: days 11-12 vs. 1-2. The final level of ethanol preference in Wistar was similar to that of P rat, and both exceeded the SD level: [df(20), $t = 1.94$, $p < 0.05$ one-tail] and [$F(1,22) = 4.75$, $p < 0.05$], respectively - Fig. 8: days 11-12.

4. Discussion

This study demonstrated immediate differences of P from both Wistar and SD rats in daily fluid intake, first in water phase, then in ethanol phase, and during the initial choice period. All groups increased ethanol intake during the choice period, but P rat showed heightened preference at the outset of choice period. Nearly identical levels of total fluid prevailed for all three groups during the choice period, suggesting an overall systemic integration of ethanol and water intake across daily cycles. Compared to other measures, a stable daily level of total fluid emerges as a main regulated parameter of fluid intake during choice conditions. A ceiling of total fluid level would effectively set an upper limit on daily ethanol intake.

4.1 Drinking of water as the sole fluid

A general challenge is to ascertain what level of fluid intake should be appropriate for a given circumstance. An initial finding is that ethanol-naïve P consumed ~20% more water than Wistar and SD rats, 65 vs. 53 – 54 ml/kg (Fig. 1: 1-ABC). Previously reported values for adult P [27], Wistar [28], and SD rats [29], in the 80-100 ml/kg range, were found under 24-hr fluid access. The lower intake in the present study may have been simply due to a shorter fluid access time.

Typical fluid intake is above levels self-administered during physiological thirst, and is thought likely to result from a contextual stimulation through cues related to anticipated need of food and water [11]. Fluid intake may overlap typical levels by as much as 200-300% under special conditions that exceed routine regulatory context of a free-behaving rat, such as scheduled access to reward [30], or the use of gustatory attractive additives [27]. Intake may be higher in certain lines [28], or in young vs. old and in female vs. male rats [31]. Such conditions do not occur in the present study, and only adult male rats of commonly used lines were tested.

Considering prandial influence on levels of daily fluid intake, we found in the literature that with concurrent food, Sardinian alcohol-preferring rats (sP) showed fluid intakes of 80–100 ml/kg under 24-hr access [32], and a level of ~120 ml/kg (reconstructed, see section 4.4) under 12-hr dark-phase access [33]. On the other hand, with food absent, the 24-hr intake of water in Wistar and SD rats [28,34] decreased to 44-42% of intake with concurrent food. That is, elimination of prandial influence, vs. dark-phase-only fluid accessibility, appears to reduce the daily fluid intake. Absence of prandial modulation is most likely a reason for our findings of lowered daily fluid intake. Water intake in our study constituted 53-81% of

levels reported elsewhere yet clearly sustained adequate hydration for normal development (see section 2.4.4).

4.2 Drinking of 10E as the sole fluid

A second finding is that with access to 10E as the sole fluid, P rats consumed more ethanol than common-stock rats, 4.5 vs. 4.1 g/kg/day - Fig. 1: 2-D. This is not a great difference, and may simply be due to a lower alcohol aversion in P vs. common-stock rats [35]. Similar levels of ethanol intake during forced ethanol exposure have been found in SD and high alcohol consuming rats [36,37].

However, our findings also appear to be related to the direct influence of ethanol on water balance. Initial ethanol exposure has been shown to lead to increased diuresis [38] and decreased hydration [26]. After 1-week exposure, intake of forced ethanol solution-bound water increased gradually [36] and water balance began to recover [25]. Tissue hydration in common stock rats was restored fully after two months [26]. Over this time, phasic adaptations were observed in vasopressin (VP) mRNA production in the paraventricular nucleus (PVN) of the hypothalamus [39,40]. VP gene expression in ethanol-free Wistar rat positively correlated with ethanol intake [41], and VP mRNA levels of ethanol-naïve P exceeded those of low alcohol-consuming rats [42].

In the ethanol phase of the present study, ethanol solution-bound water was below the water phase intake in Wistar and SD rats, corresponding to a negative water balance vs. ethanol-naïve state. However, in P rat, ethanol (10E) intake was sufficiently high to keep influx of the solution-bound water at levels of normal hydration as found for ethanol-naïve non-selected rats in the water phase - Fig. 1: 2-B vs. 1-ABC. These findings suggest a fortuitous protection of P rat at a behavioral, in addition to genomic (VP), level against the dehydrating influence of ethanol.

4.3 Ethanol, water and total fluid intake in choice conditions

During choice period, consumption of 10E and water varied, but total fluid stayed remarkably similar across groups and over time - Figs. 1: 3-A, 7. Ethanol intake for each group started in choice period below the end level of the ethanol phase (Fig. 5: days 1-2 vs. Fig. 3: day 5), indicating that total fluid levels are not linked to a specific amount of ethanol. Free water intake at the start of choice period was below vs. the end level of water phase (Fig. 4: days 1-2 vs. Fig. 2: day 5), therefore, water selection also is not specifically regulated.

Ethanol intake in P was immediately greater than in non-selected rats, but the level consumed by Wistar appeared to converge with that of P rat after ten days - Fig. 5. Similar differences of P vs. Wistar rats in starting levels and elevation rate of ethanol intake under choice conditions were demonstrated previously in a longer experiment [43]. P rat choice drinking in laboratory vs. home-cage housing conditions have yielded a finding similar to our result of a reduced (3.4 vs. 5.0 g/kg respectively) level of ethanol intake, and the difference has been attributed to an integral influence of the laboratory chamber-specific vs. home-cage housing environment [37]. In the present study, the laboratory environment is likely to have also contributed to lower levels of P rat ethanol and total fluid intake vs. those reported elsewhere. With the extension of our protocol beyond the present 12 days, P rat may approach the 5 g/kg selection criterion.

As reported in food non-deprived rats under choice conditions, steady increases of ethanol intake and preference were accompanied by a decreasing intake of free water [21,43] which resulted in levels of total fluid intake remaining stable, similar to our findings - Fig. 7. However, in food-deprived rats, during increases of ethanol intake and preference, intake of

free water appeared to be constant and resulted in levels of total fluid intake increasing along with ethanol intake [21]. Evidently, rats not deprived of food, may titrate and integrate the amount of drinking and maintain total fluid intake near a certain stable level, whereas food deprivation counteracts such ability. In the present study, all groups showed a gradual increase of ethanol intake and preference (Figs. 5, 8), and a parallel compensatory decrease of free water intake – Fig. 4. The intake of free water during choice period was lower vs. both sole-fluid phases in each group (Fig. 1: 3-C vs. 1-ABC and 2-B), and the ethanol solution-bound water was the only source to supplement the difference. The resulting levels of total fluid remained rather stable and similar in all groups during choice period (Figs. 1: 3-A; 7), serving as what appears to be an internal control and indicating that differences when they are present are not random phenomena.

4.4 Levels of total fluid intake

The similarity and relative stability of total fluid levels in P, Wistar and SD rats over the choice drinking (Figs. 1: 3-A; 7) suggest a regulated limit to total fluid intake integrated over a 24-hr cycle. Does this possibility also emerge from other reports? Total fluid levels in choice conditions when reported directly for sP, Wistar and cAA rats [32,44,45] are in the 80–100 ml/kg range. The same values can be reconstructed from the reported g/kg intake, preference, concentration of ethanol, body weight and ml/animal intake, and the specific density of pure ethanol [46], for example, based on the cut-off criteria of intake parameters for the selective breeding of P/NP (alcohol-nonpreferring) lines [47]. Indeed, the P rat intake criterion of 5 g/kg ethanol equates to ~63 ml/kg of 10E ($5 \times 10 / 0.78934$), and the 2:1 preference yields ~95 ml/kg total fluid. Similarly, in NP rat, 1.5 g/kg ethanol = ~19 ml/kg 10E, and with 0.2:1 preference total fluid is ~95 ml/kg as well. This method, for an adult male P, sP and Wistar rats weighing 375–475 g [27,35,48,49] consistently returns total fluid within the 80–100 ml/kg range, under 24-hr concurrent access to ethanol / water choice and food. The same levels were reported directly for P, Wistar and SD rats of similar weight under 24-hr concurrent access to water as the sole fluid and food, as referenced earlier.

In the present study, lower levels of total fluid were likely (see section 4.1) due to lack of prandial influence. In such conditions, P rat total fluid was at the same level during both water phase and ethanol / water choice period, while Wistar and SD intake was lower in water phase and reached the same level as P rat only in choice period – Fig. 1: 3-A vs. 1-ABC. It appears that when released from prandial influence, common stock rats, unlike P rat, drink less fluid with access to water alone vs. choice conditions. We suggest that elevated total fluid in common stock rat during choice conditions helps to counter dehydrating influence of ethanol, but that this became an inherited trait in a process of P rat selective breeding (see section 4.2). P rat has been selectively bred for high ethanol intake and preference, and so this might lead one to presume an increased motivation specific for ethanol as an exclusive basis for elevated ethanol intake. However, the motivation for taking ethanol itself in choice conditions parallels the motivation for fluid-taking based on a need to supplement the reduced intake of free water paired with the systemic maintenance of an elevated stable level of total daily fluid. This dualistic nature of the developing drive for ethanol intake, with a combination of a drug (ethanol) and natural (drinking) motivational components appears a unique feature of alcohol as one of the classic abused drugs. Where does the parallel mechanism for choosing alcohol reside? This question can be resolved through the neurophysiologic analysis of neural activity patterns specific for consumption of ethanol vs. water, the study that in turn requires a high precision analysis beyond the traditional parameters of daily intake and preference, to target consumption patterns and bouts.

4.5 Total fluid and ethanol metabolizing capacity as limits to ethanol intake

Intake of 100 ml/kg of fluid appears to be an upper limit of total daily fluid in 10E / water choice conditions for SD, Wistar and Wistar-derived high-alcohol-consuming adult male rats. A fluid volume of 100 ml may contain as much as ~8 g ethanol if all this fluid consisted of 10E. For Wistar-derived high-alcohol-consuming male rats with 24-hr concurrent access to unsweetened 10E, water and food in standard conditions, 8 g/kg emerges as an approximate maximum for daily ethanol intake during an initial month from a great number of studies. The ethanol-metabolizing capacity of an ethanol-naïve male rat amounts to ~12 micromole/100g/min in SD [50], ~7.5-8 mmol/hr/kg in Wistar [51] and ~0.33 g/kg/hr in UChA and UChB rats [52] - or ~8 g/kg/day. In the present study ethanol intake remains below the metabolic limit, leaving a possibility for blood ethanol concentration (BEC) to spike transiently and rapidly return to near zero values. Technology has not been available yet for real-time non-disturbing measurement of changing BECs in free-behaving drinking rats. The total fluid intake of P rat during choice period in the present study was ~68 ml/kg. This level may correspond to as much as ~5.4 g/kg ethanol, if the preference for 10E were ~100% ($68 \times 0.78934/10$). This would allow P rat ethanol intake to reach the 5 g/kg selection criterion during choice conditions if they were extended beyond the 12 days of the present study.

The total fluid limitation may operate even when an ethanol-experienced rat is set to override the initial metabolizing capacity. Ethanol intake has been found to increase from the baseline of 6-7 g/kg and reach 15-18 g/kg when the alcohol-deprivation effect (ADE) developed under concurrent access to 10% and more concentrated 20% and 30% ethanol solutions [53,54]. According to our estimates, the reconstructed levels of total fluid in those conditions remained close to 100 ml/kg during both baseline and ADE. Thus, the role of more concentrated solutions appears to allow fitting more ethanol into the same limited volume of total fluid. The potential for antidipsic interference to reduce ethanol drinking has received attention previously [55]. Our findings support testing of antidipsic compounds to reduce total fluid intake as one of the promising future directions [1] in search of medications for alcohol abuse.

4.6 Conclusions

The total fluid intake during choice drinking emerges as a uniformly regulated and stable parameter, subject to a common limit across the P, Wistar and SD rats when the major modulation from prandial and environmental factors is controlled by the design of the protocol. This regulated parameter sets an effective limit to ethanol intake in choice conditions. Behavioral differences between P and non-selected rats appear immediately in each phase of the study. P rats show higher intake of water as the sole fluid. Intake in P rat is also higher with access to 10E as the sole fluid, where their solution-bound water is at a normal hydration level of ethanol-naïve non-selected rats. In choice conditions, P rats exhibit the highest ethanol intake at the outset. The similar end levels of daily ethanol intake and preference in P and Wistar rats suggest that these parameters are not universally adequate for behavioral separation of genetically different groups of alcohol-consuming rats. Drinking is known to fluctuate markedly during a day, while daily measured levels are integrated values that neglect magnitude and timing of drinking fluctuations and consumed volumes during these fluctuations. The present study indicates a need to conduct a micro-behavioral analysis of ethanol and water consumption, which is the focus of the concurrent report where further similarities and distinctions between P, Wistar, and SD rats are discussed.

REFERENCES

- [1]. Bell RL, Sable HJ, Colombo G, Hyytia P, Rodd ZA, Lumeng L. Animal models for medications development targeting alcohol abuse using selectively bred rat lines: Neurobiological and pharmacological validity. *Pharmacol Biochem Behav.* 2012; 103:119–55. [PubMed: 22841890]
- [2]. Bell RL, Rodd ZA, Lumeng L, Murphy JM, McBride WJ. The alcohol-preferring P rat and animal models of excessive alcohol drinking. *Addict Biol.* 2006; 11:270–88. [PubMed: 16961759]
- [3]. Cicero, TJ. A critique of animal analogues of alcoholism. In: Majchrowicz, E.; Noble, EP., editors. *Biochemistry and pharmacology of ethanol.* Vol. 2. Plenum Press; New York: 1979. p. 533–60.
- [4]. Lester D, Freed EX. Criteria for an animal model of alcoholism. *Pharmacol Biochem Behav.* 1973; 1:103–7. [PubMed: 4204511]
- [5]. McBride WJ, Li T-K. Animal models of alcoholism: neurobiology of high alcohol-drinking behavior in rodents. *Crit Rev Neurobiol.* 1998; 12:339–69. [PubMed: 10348615]
- [6]. Kampov-Polevoy AB, Matthews DB, Gause L, Morrow AL, Overstreet DH. P rats develop physical dependence on alcohol via voluntary drinking: changes in seizure thresholds, anxiety, and patterns of alcohol drinking. *Alcohol Clin Exp Res.* 2000; 24:278–84. [PubMed: 10776663]
- [7]. Lumeng, L.; Hawkins, TD.; Li, T-K. New strains of rats with alcohol preference and non-preference. In: Thorman, RG.; Williamson, JR.; Drott, H.; Chance, B., editors. *Alcohol and Aldehyde Metabolizing Systems.* Vol. 3. Academic Press; New York: 1977. p. 537–44.
- [8]. Azarov AV, Woodward DJ. Ethanol access mode affects initial patterns of intake in P rat. *Society for Neuroscience Abstracts.* 2006 #194.6.
- [9]. Azarov AV, Woodward DJ. Early manifestation of alcohol deprivation effect in P rat. *Alcohol Clin Exp Res.* 2010; 34(6, suppl. to June):82A. #286.
- [10]. Woodward DJ, Azarov AV. Coding within cortico-mesolimbic neurons related to early ethanol drinking in P rat. *Alcohol Clin Exp Res.* 2007; 31(6, suppl. to June):143A. #537.
- [11]. Rolls, BJ.; Rolls, ET. *Thirst.* Cambridge University Press; Cambridge: 1982.
- [12]. Fitzsimons TJ, Le Magnen J. Eating as a regulatory control of drinking in the rat. *J Comp Physiol Psychol.* 1969; 67:273–83. [PubMed: 5787378]
- [13]. Kissileff HR. Food-associated drinking in the rat. *J Comp Physiol Psychol.* 1969; 67:284–300. [PubMed: 5787379]
- [14]. Samson HH, Tolliver GA, Pfeffer AO, Sadeghi K, Haraguchi M. Relation of ethanol self-administration to feeding and drinking in a nonrestricted access situation in rats initiated to self-administer ethanol using the sucrose-fading technique. *Alcohol.* 1988; 5:375–85. [PubMed: 3219185]
- [15]. Demaria-Pesce VH, Nicolaïdis S. Mathematical determination of feeding patterns and its consequence on correlational studies. *Physiol Behav.* 1998; 65:157–70. [PubMed: 9811378]
- [16]. Glendinning JI, Smith JC. Consistency of meal patterns in laboratory rats. *Physiol Behav.* 1994; 56:7–16. 1994. [PubMed: 8084910]
- [17]. Zorrilla EP, Inoue K, Fekete EM, Tabarin A, Valdez GR, Koob GF. Measuring meals: structure of prandial food and water intake of rats. *Am J Physiol Regul Integr Comp Physiol.* 2005; 288:R1450–67. [PubMed: 15637168]
- [18]. Gauvin DV, Moore KR, Holloway FA. Do rat strain differences in ethanol consumption reflect differences in ethanol sensitivity or the preparedness to learn? *Alcohol.* 1993; 10:37–43. [PubMed: 8447965]
- [19]. Khanna JM, Kalant H, Shah G, Sharma H. Comparison of sensitivity and alcohol consumption in four outbred strains of rats. *Alcohol.* 1990; 7:429–34. [PubMed: 2222846]
- [20]. Carroll ME, Meisch RA. Determinants of increased drug self-administration due to food deprivation. *Psychopharmacology (Berl).* 1981; 74:197–200. [PubMed: 6115446]
- [21]. Marcucella H, Munro I. Patterns of ethanol and water consumption as a function of restricted ethanol access and feeding condition. *Psychopharmacology (Berl).* 1986; 89:145–9. [PubMed: 3088628]
- [22]. Gatto GJ, Murphy JM, Waller MB, McBride WJ, Lumeng L, Li T-K. Chronic ethanol tolerance through free-choice drinking in the P line of alcohol-preferring rats. *Pharmacol Biochem Behav.* 1987; 28:111–5. [PubMed: 3659102]

- [23]. Guccione L, Djouma E, Penman J, Paolini AG. Calorie restriction inhibits relapse behaviour and preference for alcohol within a two-bottle free choice paradigm in the alcohol preferring (iP) rat. *Physiol Behav.* 2013; 110-111:34–41. [PubMed: 23246223]
- [24]. Murphy JM, McBride WJ, Lumeng L, Li T-K. Monoamine and metabolite levels in CNS regions of the P line of alcohol-preferring rats after acute and chronic ethanol treatment. *Pharmacol Biochem Behav.* 1983; 19:849–56. [PubMed: 6196794]
- [25]. Crow LT. Water metabolism with prolonged ethanol consumption in the rat. *Psychol Rep.* 1970; 27:675–7. [PubMed: 5485538]
- [26]. Wright JW, Donlon K. Reversal of ethanol induced body dehydration with prolonged consumption in rats. *Pharmacol Biochem Behav.* 1979; 10:31–6. [PubMed: 441092]
- [27]. Overstreet DH, Halikas JA, Seredenin SB, Kampov-Polevoy AB, Viglinskaya IV, Kashevskaya O, et al. Behavioral similarities and differences among alcohol-preferring and -nonpreferring rats: confirmation by factor analysis and extension to additional groups. *Alcohol Clin Exp Res.* 1997; 21:840–8. [PubMed: 9267533]
- [28]. Walsh LL. Differences in food, water and food-deprivation water intake in 16 strains of rats. *J Comp Physiol Psychol.* 1980; 94:775–81.
- [29]. He J, Varma A, Weissfeld LA, Devaskar SU. Postnatal glucocorticoid exposure alters the adult phenotype. *Am J Physiol Regul Integr Comp Physiol.* 2004; 287:R198–208. [PubMed: 15001431]
- [30]. Falk JL. The nature and determinants of adjunctive behavior. *Physiol Behav.* 1971; 6:577–88. [PubMed: 5004684]
- [31]. Bell RL, Rodd ZA, Sable HJ, Schultz JA, Hsu CC, Lumeng L, et al. Daily patterns of ethanol drinking in peri-adolescent and adult alcohol-preferring (P) rats. *Pharmacol Biochem Behav.* 2006; 83:35–46. [PubMed: 16442608]
- [32]. Colombo G, Agabio R, Carai MA, Lobina C, Pani M, Reali R, et al. Ability of baclofen in reducing alcohol intake and withdrawal severity: I-Preclinical evidence. *Alcohol Clin Exp Res.* 2000; 24:58–66. [PubMed: 10656194]
- [33]. Balakleevsky A, Colombo G, Fadda F, Gessa GL. Ro 19-4603, a benzodiazepine receptor inverse agonist, attenuates voluntary ethanol consumption in rats selectively bred for high ethanol preference. *Alcohol Alcohol.* 1990; 25:449–52. [PubMed: 1965120]
- [34]. Rowland NE, Fregly MJ. Behavioral and physiological aspects of body fluid homeostasis in Fischer 344 rats. *Physiol Behav.* 1988; 42:499–505. [PubMed: 3413226]
- [35]. Rezvani AH, Sexton H, Levin ED. Persistent high alcohol consumption in alcohol-preferring (P) rats results from a lack of normal aversion to alcohol. *Alcohol Alcohol.* 2010; 45:219–22. [PubMed: 20356869]
- [36]. Aradottir S, Lundqvist C, Alling C. Phosphatidylethanol in rat organs after ethanol exposure. *Alcohol Clin Exp Res.* 2002; 26:514–8. [PubMed: 11981128]
- [37]. Timberlake W, Leffel JK, Chester JA, Froehlich JC. Effects of forced alcohol drinking on alcohol-water choice in three pairs of rat lines selectively bred for differences in alcohol preference. *Alcohol.* 2009; 43:105–18. [PubMed: 19251112]
- [38]. Rubini ME, Kleeman CR, Lamdin E. Studies on alcohol diuresis. I. The effect of ethyl alcohol ingestion on water, electrolyte and acid-base metabolism. *J Clin Invest.* 1955; 34:439–47. [PubMed: 14354014]
- [39]. Madeira MD, Paula-Barbosa MM. Effects of alcohol on the synthesis and expression of hypothalamic peptides. *Brain Res Bull.* 1999; 48:3–22. [PubMed: 10210163]
- [40]. Resstel LB, Scopinho AA, Lopes da Silva A, Antunes-Rodrigues J, Corrêa FM. Increased circulating vasopressin may account for ethanol-induced hypertension in rats. *Am J Hypertens.* 2008; 21:930–5. [PubMed: 18464746]
- [41]. Pickering C, Avesson L, Liljequist S, Lindblom J, Schiöth HB. The role of hypothalamic peptide gene expression in alcohol self-administration behavior. *Peptides.* 2007; 28:2361–71. [PubMed: 17976860]
- [42]. Hwang BH, Froehlich JC, Hwang WS, Lumeng L, Li T-K. More vasopressin mRNA in the paraventricular hypothalamic nucleus of alcohol-preferring rats and high alcohol-drinking rats

- selectively bred for high alcohol preference. *Alcohol Clin Exp Res.* 1998; 22:664–9. [PubMed: 9622448]
- [43]. Simms JA, Steensland P, Medina B, Abernathy KE, Chandler LJ, Wise R, et al. Intermittent access to 20% ethanol induces high ethanol consumption in Long-Evans and Wistar rats. *Alcohol Clin Exp Res.* 2008; 32:1816–23. [PubMed: 18671810]
- [44]. Ericson M, Engel JA, Söderpalm B. Peripheral involvement in nicotine-induced enhancement of ethanol intake. *Alcohol.* 2000; 21:37–47. [PubMed: 10946156]
- [45]. Maurel S, De Vry J, De Beun R, Schreiber R. 5-HT_{2A} and 5-HT_{2C/5-HT_{1B}} receptors are differentially involved in alcohol preference and consummatory behavior in cAA rats. *Pharmacol Biochem Behav.* 1999; 62:89–96. [PubMed: 9972850]
- [46]. Washburn, EW., editor. International critical tables of numerical data, physics, chemistry and technology. 1st ed. Vol. 3. McGraw-Hill Book Company; New York: 1928. p. 27 editor-in-chief
- [47]. Li TK, Lumeng L, Doolittle DP. Selective breeding for alcohol preference and associated responses. *Behavior Genetics.* 1993; 23:163–70. [PubMed: 8099788]
- [48]. Colombo G, Lobina C, Carai MA, Gessa GL. Phenotypic characterization of genetically selected Sardinian alcohol-preferring (sP) and -non-preferring (sNP) rats. *Addict Biol.* 2006; 11:324–38. [PubMed: 16961762]
- [49]. Wolffgramm J. Free choice ethanol intake of laboratory rats under different social conditions. *Psychopharmacology (Berl).* 1990; 101:233–9. [PubMed: 2349365]
- [50]. Eriksson CJ, Sippel HW. The distribution and metabolism of acetaldehyde in rats during ethanol oxidation-I. The distribution of acetaldehyde in liver, brain, blood and breath. *Biochem Pharmacol.* 1977; 26:241–7. [PubMed: 843394]
- [51]. Lumeng L, Waller MB, McBride WJ, Li TK. Different sensitivities to ethanol in alcohol-preferring and -nonpreferring rats. *Pharmacol Biochem Behav.* 1982; 16:125–30. [PubMed: 7199181]
- [52]. Quintanilla ME, Israel Y, Sapag A, Tampier L. The UChA and UChB rat lines: metabolic and genetic differences influencing ethanol intake. *Addict Biol.* 2006; 11:310–23. [PubMed: 16961761]
- [53]. Rodd ZA, Bell RL, Kuc KA, Murphy JM, Lumeng L, McBride WJ. Effects of concurrent access to multiple ethanol concentrations and repeated deprivations on alcohol intake of high-alcohol-drinking (HAD) rats. *Addict Biol.* 2009; 14:152–64. [PubMed: 19076927]
- [54]. Rodd-Henricks ZA, Bell RL, Kuc KA, Murphy JM, McBride WJ, Lumeng L, et al. Effects of concurrent access to multiple ethanol concentrations and repeated deprivations on alcohol intake of alcohol-preferring rats. *Alcohol Clin Exp Res.* 2001; 25:1140–50. [PubMed: 11505045]
- [55]. Koob GF, Roberts AJ, Kieffer BL, Heyser CJ, Katner SN, Ciccocioppo R, et al. Animal models of motivation for drinking in rodents with a focus on opioid receptor neuropharmacology. *Recent Dev Alcohol.* 2003; 16:263–81. [PubMed: 12638642]

Highlights

- Initial development of alcohol and water intake in P vs. nonselected rats.
- Intake volumes and timing measured precisely, prandial confounds eliminated.
- P rats consume more water when alcohol naïve and more ethanol in forced drinking.
- P vs. nonselected rat EtOH intake in choice is the highest, but total fluid the same.
- Total fluid is the main regulated parameter in choice and a limit to EtOH intake.

Fig. 1.

Intake levels over phases of the experiment in P, Wistar (W) and Sprague Dawley (SD) rats. **A:** total fluid – of water phase (1-ABC) in P was higher vs. W, SD a combined W+SD rats. Each group reduced total fluid from water phase (1-ABC) to ethanol phase (2-A) and from there increased during choice period (3-A). In choice conditions, total fluid of W and SD was at the highest level, and in all groups was equal to the water phase level of P rat. **B:** total water – of P in the ethanol phase (2-B) was at the water phase level (1-ABC) of non-selected rats. Each group reduced total water from the water (1-ABC) to ethanol (2-B) phase and from there increased during the choice period (3-B). In choice conditions, total water of W and SD rats was at the highest level. **C:** free water – of each group in choice conditions (3-C) was lower vs. the water level in both sole-fluid phases (1-ABC, 2-B). **D:** ethanol intake – of P was higher vs. non-selected rats during both the ethanol phase (2-D) and the choice period (3-D). Each group reduced ethanol intake from ethanol phase to choice conditions. The values represent means $\pm$ SEM. Differences significant at $p < 0.05$ or less are indicated: + – between P and W, x – between P and SD, * – between W and SD, Σ – between P and averaged W+SD rats, for a particular parameter, during a given phase; < – between a particular and a preceding phase, v – between free water in choice (3-C) and total water in sole-fluid phases (1-ABC, 2-B), for a given group.

Fig. 2. Intake of water as the sole fluid in P, Wistar (W) and Sprague Dawley (SD) rats fluctuated daily and showed slight tendency to decrease. On the day 5, the P water intake was above that of non-selected rats. The values represent means $\pm$ SEM. Symbols are as per the legend to Fig. 1.

Fig. 3. Intake of ethanol (10% v/v solution, 10E) as the sole fluid in P, Wistar (W) and Sprague Dawley (SD) rats fluctuated daily. P and W tended to increase, and SD rat - to decrease ethanol intake. On the day 5, the P ethanol intake was greater vs. non-selected rats. The values represent means $\pm$ SEM. Symbols are as in the legend to Fig. 1.

Fig. 4.

Intake of free water in P, Wistar (W) and Sprague Dawley (SD) rats during the choice period. On days 1-2 in every group, water intake was significantly lower vs. the day 5 of water phase (Fig. 2, day 5). From the days 1-2 to 11-12, intake decreased significantly in each group. On the days 1-2, the P intake was below the other rats, by the days 11-12 the SD intake remained the highest. The values represent means $\pm$ SEM. Differences significant at $p < 0.05$ or less are indicated by: - between the days 1-2 of choice period and the day 5 of a respective sole-fluid phase (Fig. 2 or 3); ^ - between the days 11-12 and 1-2; other symbols are as per the legend to Fig. 1.

Fig. 5. Ethanol (10% v/v solution, 10E) intake in P, Wistar (W) and Sprague Dawley (SD) rats during the choice period. On days 1-2 in every group, ethanol intake was significantly lower vs. the day 5 of ethanol phase (Fig. 3, day 5). From the days 1-2 to 11-12, intake increased significantly in non-selected, and to days 11-12, ethanol intake in W approached the level of P rat, and that of SD remained below the other rats. The values represent means $\pm$ SEM. Symbols are as per the legend to Fig. 4.

Fig. 6. Total water intake in P, Wistar (W) and Sprague Dawley (SD) rats - at the start of choice period was similar. During choice period, total water intake in each group remained similar to a common final level of ~61 ml/kg. The values represent means $\pm$ SEM. Symbols are as per the legend to Fig. 4.

Fig. 7. Total fluid intake in P, Wistar (W) and Sprague Dawley (SD) rats during the choice period - appeared very similar between groups and close to a nearly identical level of ~65 ml/kg on days 11-12, suggesting that total fluid intake is integrated over 24-hour cycle and regulated uniformly in all groups. The values represent means $\pm$ SEM. Symbols are as per the legend to Fig. 4.

Fig. 8. Ethanol preference in P, Wistar (W) and Sprague Dawley (SD) rats during the choice period. days 1-2, ethanol preference in P was the highest vs. non-selected rats. From the days 1-2 to 11-12, preference increased significantly in each group. By the days 11-12, ethanol preference in W approximated the level in P rat, while in SD it remained the lowest vs. other rats. The values represent means $\pm$ SEM. Symbols are as per the legend to Fig. 4.